Technische Universiteit
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Biomedical Engineering

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Colony PCR

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Colony PCR

1 Colony picking

- Using a pipette, pick a number (4-6) colonies and put them into an epp with 15 µL H_2O.

2 PCR

- Construct the PCR mixture in the following way:

Component	Quantity/mass/final concentration	Volume (µL)
DNA (from bacteria)	*Pipette tip in 15 µL H2O*	1
2x KAPA2G mix	1x	12.5
Primer FW	0.5 µM (stock: 10 µM)	1.25
Primer RV	0.5 µM (stock: 10 µM)	1.25
H_2O		9
Total		25

Total mastermix

Component	Quantity/mass/final concentration	Volume (µL)
DNA (from bacteria)	Pipette tip in 15 µL H_2O	1 for each separate PCR mixture
2x KAPA2G mix	1x	62.5
Primer FW	0.5 µM (stock: 10 µM)	6.25
Primer RV	0.5 µM (stock: 10 µM)	6.25
H_2O		35
Total		125

- Run PCR in the PCR machine with the following protocol:

Step	Temperature (°C)	Time (sec)	Cycles
Denaturation	95	180 (3 min)	1
Denaturation Annealing Extension	95 55-60 72	15 15 20 sec/kb	35
Final extension	72	600 (10 min)	1
Cooling	4	Hold	1

3 Gel electrophoresis

- Once the colony PCR has been completed the samples (20 µL; first mix with 4 µL loading dye) should be loaded on to an electrophoresis gel and run.

After colony PCR, perform plasmid amplification of the vectors that are correct.